

DEPARTMENT OF HEALTH AND HUMAN SERVICES

NOTE TO FILE (BNF0033)

March 25, 1996

Subject: Colorado Potato Beetle (CPB)- Resistant Potato

Keywords:

Potato, "NewleafTM" varieties Atlantic and Superior, Colorado Potato Beetle, *Leptinotarsa decemlineata*, *Bacillus thuringiensis* var. *tenebrionis*, *cryIIIA*, insect resistance, Btt protein, *kanR* (*nptII*), kanamycin resistance, APH(3')II protein (NPTII protein).

Background

In a submission dated January 24, 1996, Monsanto Company provided summary information to support their safety and nutritional assessment of their new potato varieties, which included five genetically modified Colorado Potato Beetle (*Leptinotarsa decemlineata*) (CPB)- resistant, NewleafTM, variety Atlantic (transformation events ATBTO4 -6, -27, -30, -31, and -36), and two NewleafTM, variety Superior (transformation events SPBTO2-5 and -7), lines. This submission parallels Monsanto's previous summary (cross reference FDA file no. BNF005), concerning the development of seven lines of CPB-resistant NewleafTM, variety Russet Burbank, potatoes.

Intended Effect and Food/Feed Use

The intended technical effect of the genetic modification of potato plants is to confer resistance to lepidopteran insects, specifically the Colorado Potato Beetle (CPB) (*Leptinotarsa decemlineata*).

According to Monsanto, their genetically engineered potato lines have been modified to express a synthetic version of the *cryIIIA* gene, which is similar to the *cryIIIA* gene isolated from *Bacillus thuringiensis* var *tenebrionis*. The *cryIIIA* gene encodes the Btt protein, which is toxic to certain lepidopteran insects upon ingestion. Specifically, a truncated, but biologically active, version of the Btt protein ("Btt band 3 protein") is expressed by Monsanto's CPB-resistant potato lines.

According to Monsanto, their genetically engineered potato lines have been modified to express the *kanR* (*nptII*) gene, which is derived from the prokaryotic transposon *Tn* 5. The *kanR* gene encodes the enzyme neomycin phosphotransferase II (APH(3')II) (NPTII), which renders plants resistant to aminoglycoside antibiotics. Monsanto used this insert as a marker gene for early in vitro selection of *cryIIIA*-transformed potato plants.

Molecular Alterations and Characterization

Monsanto reported using the *Agrobacterium*-mediated plant transformation system to incorporate T-DNA from two plasmid vector constructs, designated PV-STBT02 and PV-STBT04, into potato plants. These plasmid vectors contain commonly used bacterial selection and replication elements as well as T-DNA right and left borders, which delineate the DNA to be transferred into the potato plant genome; hence, they are termed double border transformation vectors. Monsanto stated that, in general, for double border vector plant transformation systems, the DNA transferred and integrated into the plant genome is confined to the DNA within the border regions, i.e., the T-DNA, and that the T-DNA is integrated in an irreversible manner. Occasionally, insertion of plasmid DNA located outside the T-DNA may occur.

According to Monsanto: "[t]wo chimeric genes, with signals for plant expression, were introduced between the right and left border regions of the vectors PV-STBT02 and PV-STBT04. Both plasmid vectors contain a chimeric gene for selection on kanamycin." Both plasmid vectors also contain a second chimeric gene, responsible for efficacious control of CPB. The nature of this latter insert, which employs alternative promoters, distinguishes the two vectors. In other words, PV-STBT02 and PV-STBT04 "differ only in the nontranslated promoter region of the *cryIIIA* gene."

Monsanto stated that plasmid vector PV-STBT02 was used to develop the two new NewleafTM, variety Superior lines, designated SPBT02-5 and -7, and plasmid vector PV-STBT0-4 was used to develop the five new NewleafTM, variety Atlantic lines, designated ATBT04-6, -27, -30, -31, and -36. All developed lines contain the *cryIIIA* and *kanR* genes. Three of these lines also contain DNA sequences located in PV-STBT02 and PV-STBT04 that are outside the T-DNA borders.

Regulatory Considerations

The use of Btt protein, a pesticidal substance, is under the Environmental Protection Agency's (EPA) regulatory purview. For Monsanto's development of CPB-resistant potatoes, the APH(3')II (NPTII) protein is used as a plant pesticide inert ingredient; therefore, such use is regulated by EPA according to 59 FR 49351 (Federal Register, 28 September 1994). For this reason, FDA limits its consideration of Monsanto's notification regarding CPB-resistant potatoes to composition and wholesomeness of the varieties they have developed.

Nutritional Assessment

Monsanto conducted compositional analyses of their Newleaf™ potato lines and showed that potato tubers derived from the two new Superior lines were substantially equivalent to tubers derived from control Superior tubers presently in commerce. Constituents analyzed included total solids, sugars, vitamin C, protein, and naturally occurring glycoalkaloid toxicants. Monsanto reported that "except for a statistically significant lower level of solids in line SPBT02-7, no statistically significant differences were observed between any CPB-resistant line and Superior control line for any of the analyzed parameters."

Monsanto conducted compositional analyses on their Atlantic lines and found statistically significant differences in the level of dextrose for lines ATBT04-30, -31, and -36 as compared to control Atlantic tubers presently in commerce. A statistically significant difference in the level of sucrose was also observed for line ATBT04-30 when compared to controls. According to Monsanto, large variations in these type of analyses are common and attributable to agronomic practices. Monsanto also stated that a significantly lower level of total glycoalkaloid was observed for line ATBT04-36, and that this level fell within the range for potatoes in commerce. Therefore, Monsanto concluded that their Atlantic varieties were substantially equivalent to tubers derived from control Atlantic potato tubers presently in commerce.

Based on qualitative and quantitative evaluation of the major constituents of their new genetically engineered potatoes and parental controls, Monsanto concluded that the Newleaf™ Superior and Atlantic potato lines have not been significantly altered within the meaning of 21 CFR 170.30(f)(2).

Conclusions

Monsanto has concluded that potato lines containing transformation events ATBT04-6, -27, -30, -31, and -36 and STBT02-5 and -7 are not materially different in composition, nutrition, and safety from potatoes currently grown, marketed, and consumed for human food. At this time, based on Monsanto's description of its data and analyses, the Agency considers Monsanto's consultation on the aforementioned genetically modified potatoes to be complete.



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